The role of Blastocystis hominis *in the activation of ulcerative colitis*

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ABSTRACT

Background/Aims: *Several studies have shown that a change in microbiota plays an important role in the pathogenesis of inflammatory bowel disease (IBD). Furthermore, with the emergence in recent studies of differences according to the subtype of IBD and whether the disease is active or in remission, there has started to be research into the relationship between IBD and several microorganisms.* Blastocystis hominis *is primary among these organisms. The aim of the present study was to determine the role of* B. hominis *in the acute flare-up of ulcerative colitis (UC).*

Materials and Methods: *A total of 114 patients with UC were included in the study, with 52 in the active phase. The Mayo scoring system was used for the activity index. Patients determined with a flare-up agent other than* B. hominis *were excluded from the study. Fecal samples of the patients were examined by the polymerase chain reaction method for the presence of* B. hominis.

Results: B. hominis *positivity was determined in 37 (34%) patients with UC. Of the patients, 17 (32.6%) were in the acute flare-up phase, and 20 (32.2%) were in remission (p=0.961). In 11 (64.7%) of the* B. hominis *positive patients, the disease severity was determined as mild-moderate (p<0.001).*

Conclusion: *The results of the present study showed that while there was no difference between the active and remission phases in respect of B.* hominis *presence, there was milder involvement in those determined with* B. hominis.

Keywords: Blastocystis hominis*, ulcerative colitis, microbiota, flare-up*

INTRODUCTION

Although inflammatory bowel disease (IBD) etiology is not fully known, it is a chronic, inflammatory disease with a course of intermittent exacerbations and is thought to develop as a result of the interaction of genetic, immune system, and environmental factors (1-3). In studies investigating the pathogenesis of IBD, where the incidence in monozygote twins has been determined at <50%, environmental factors have been shown to play a more important role (4). Of the environmental factors, more emphasis has recently been focused on microbiota.

The intestinal microbiota, which is formed of bacteria, viruses, and eukaryotes (e.g., protozoa, helminths, and fungi), plays an important role in host metabolism and the immune system (5). Correspondingly, the microbiota needs the host for life. An impairment in this symbiotic relationship between the microbiota and the host leads to immune dysregulation and can cause chronic inflammation, such as IBD. Many studies have shown the role of the microbiota in the pathogenesis of IBD (2,4). Previous animal experimental studies have demonstrated that colitis did not develop in a sterile environment but developed after bacterial colonization (6,7). Moreover, colitis has been shown to develop in healthy rats following the administration of the microbiota of rats with colitis (8).

Studies of microbiota have increased in recent years with the use of new-generation sequence analyses, such as 16 sRNA sequencing (9). As a result of these studies, six major bacterial classifications, namely, Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, Fusobacteria, and Verrucomicrobia, have been identified in the intestinal microbiota of healthy humans (10). Bacteroidetes and Firmicutes have been determined to constitute 90% of the microbiota (2). The microbiota in patients with IBD has been shown to consist mainly of Actinobacteria and Proteobacteria, and a small part is formed of Firmicutes (especially *Clostridium* IX and VI groups) and Bacteroidetes (2). Although studies have shown that bacteria play a major role in the microbiota, parasites have also been shown

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recently to be necessary for a healthy microbiota (11). With regard to this subject, most focus has been on *Blastocystis*. There is a relationship between a richer bacteria content and the presence of *Blastocystis*, and as in patients with IBD, Bacteroidetes and Clostridium IX have been determined to be decreased in the microbiota of *Blastocystis* positive patients (11). As *Blastocystis hominis* has been shown to have significant effects on the intestinal microbiota, it has been associated with several diseases, such as colitis, irritable bowel syndrome, hemorrhagic proctosigmoiditis, and chronic urticaria (12).

In a previous study in our center, the prevalence of *B. hominis* was found to be higher in patients with ulcerative colitis (UC) than in the healthy control group (patients with UC=8.7% vs. control group=3.1%, p=0.016), suggesting that *B. hominis* could play a role in the pathogenesis of UC (12). However, as *B. hominis* is frequently seen in healthy people, and because in the majority of studies conducted on *B. hominis*, there has been an insufficient number of patients identified with the disease, definitive exclusion has not been made of infections other than *B. hominis*, and there have been differences in diagnostic methods, resulting in continued pathogenic uncertainty of *B. hominis* (12).

Is *B. hominis* a real innocent bystander or not? To answer this question, the present study was designed to evaluate the role of *B. hominis* in UC flare-ups.

MATERIALS AND METHODS

Patients who presented at the Gastroenterology Clinic of Antalya Training and Research Hospital between May 2014 and May 2016, who were diagnosed with UC acute flare-up, or who were being followed up in the remission period were included in the study. All the patients were aged >18 years and were diagnosed with UC by clinical, endoscopic, and pathological methods. Patients with a non-definitive diagnosis, with an intestinal disease other than UC (e.g., Crohn's disease (CD), microscopic colitis, malignancy, and infectious colitis), or who had been using antibiotics or probiotics in the previous 6 months were excluded from the study.

Fecal samples from all the patients included in the study were examined by the same specialist parasitologist using microscopy and culture. Samples of patients who presented with acute flare-up were examined by polymerase chain reaction (PCR) for *Clostridium difficile* toxins A and B, *Entamoeba histolytica* adhesion antigen, and Cytomegalovirus in the serum. Patients who were taking drugs (antibiotics and nonsteroidal anti-inflammatory drugs) that could have triggered a flare-up; those determined with infections, such as *C. difficile* toxins A and B, *E. histolytica* adhesion antigen, or Cytomegalovirus; and those who had stopped taking medication or were taking it irregularly were also excluded from the study.

The Mayo scoring system was used to evaluate UC activity. A Mayo score of 0-2 was classified as remission, 3-5 as mild flare-up, 6-10 as moderate, and >10 as severe flare-up. The ethics committee of our hospital approved the study. Genomic DNA of *Blastocystis* isolates was directly extracted from stool samples stored at -20°C using the Exgene™ stool DNA mini kit (GeneAll; Seoul, South Korea) according to the manufacturer's protocol. The real-time PCR reactions were performed on the CFX96 real-time PCR detection system (CFX96; Bio-Rad, Hercules, CA, USA). The DNA samples were subjected to PCR amplification using the primers shown in Table 1. PCR reaction mixtures (20 µL of total volume) consisted of 1× real-time PCR master mix (GenMark, Turkey), 1 M of each primer, 300 nM probe (*Blastocystis*), and DNA sample. The PCR reactions consisted of 1 cycle initial denaturing at 95°C for 15 min, 45 cycles including denaturing at 95°C for 15 s, and 1 cycle annealing at 60°C for 1 min. The *B. hominis* Brumpt (subtype 1, ATCC®50752™) strain from the American Type Culture Collection was used as the reference strain in the present study.

Statistical analysis

Descriptive statistics are presented as frequency (n), percentage (%), median (minimum-maximum), and mean±standard deviation. The Fisher's exact test and Pearson's chi-square test were used to assess the relationships between categorical variables. Conformity to normality of distribution was tested using the Shapiro-Wilk test in groups of sample size ≤50 and using

Table 1. Primers used for PCR amplification

Primer name	
Blasto FWD F5	GGTCCGGTGAACACTTTGGATTT 1641-1663 in AY244621
Blasto R F2	CCTACGGAAACCTTGTTACGACTTCA 1734-1759 in AY244621
Blastocystis probe FAM	TCGTGTAAATCTTACCATTTAGAGGA-BHQ1 1705-1730 in AY244321b

Table 2. Demographic, clinical, and biochemical parameters of the patients

		n=114
Age (years), mean±SD		43±13
Gender, n (%)	Male	64 (56.1)
	Female	50 (43.9)
Disease status, n (%)	Remission	62 (54.4)
	Active	52 (45.6)
	Pancolitis	41 (36)
Site of involvement, n (%)	Left colon	51 (44.7)
	Proctitis	22 (19.3)
	Mild	5 (9.6)
Total Mayo score, n (%)	Moderate	29 (55.8)
	Severe	18 (34.6)
B. hominis, n (%)	Positive	37 (32.5)
	Negative	77 (67.5)
Hemoglobin (g/dL), mean±SD		12.8±2.2
Eosinophil (10^3/mm^3), median (min-max)		200 (0-1100)
CRP (mg/L), median (min-max)		4 (0-216)

CRP: C-reactive protein

the Kolmogorov-Smirnov test in groups of sample size >50. The difference between the two groups was analyzed using the Mann-Whitney U test and Student's t-test where appropriate. The Kruskal-Wallis with Bonferroni-Dunn post hoc test was used to compare differences between the three groups with non-normal distribution. A p value of <0.05 was considered to be statistically significant. All analyses were performed using Statistical Package for Social Sciences version 22.0 (IBM Corp.; Armonk, NY, USA).

RESULTS

A total of 114 patients with UC were included in the study. There were 64 (56.1%) male and 50 (43.9%) female patients. The mean age of the patients was 43±13 years. Of the patients, 52 (45.6%) were in the acute flare-up phase. According to the Montreal classification, proctitis was determined in 22 (19.3%), left colon involvement in 51 (44.7%), and pancolitis in 41 (36%) patients. According to the Mayo scoring system, the severity of the involvement was determined as mild in 5 (9.6%), moderate in 29 (55.8%), and severe in 18 (34.6%) patients. With the PCR method, *B. hominis* was determined in 37 (32.5%) patients, with 17 in the acute flare-up period. Although this was not a prevalence study, the prevalence of *B. hominis* in all patients with UC was determined as 32.5%. In laboratory analyses, mean hemoglobin was 12.8±2.2 g/dL, median eosinophil count was 200 (0-1100) 10^3/mm^3, and median C-reactive protein (CRP) was 4 (0-216) mg/L (Table 2).

Table 3. The relationship between the presence of *B. hominis* and demographic, clinical, and biochemical parameters

		B. hominis		
		Negative (n=77)	Positive (n=37)	p
Age (years), mean±SD		41.57±13.1	44.49±11.3	0.246
Gender, n (%)	Male	45 (58.4)	19 (51.4)	0.475
	Female	32 (41.6)	18 (48.6)	
Disease status, n (%)	Remission	42 (54.5)	20 (54.1)	0.961
	Active	35 (45.5)	17 (45.9)	
	Pancolitis	27 (35.1)	14 (37.8)	0.268
Site of involvement, n (%)	Left colon	32 (41.6)	19 (51.4)	
	Proctitis	18 (23.4)	4 (10.8)	
Total Mayo score, n (%)	Mild-moderate	23 (65.7)	11 (64.7)	0.943
	Severe	12 (34.3)	6 (35.3)	
Hemoglobin (g/dL), mean±SD		4 (0-216)	6 (0-104)	0.060
Eosinophil (10^3/mm^3), median (min-max)		13±2.2	12.4±2.2	0.165
CRP (mg/L), median (min-max)		200 (0-1100)	100 (0-800)	0.965

CRP: C-reactive protein

Table 4. Comparisons of the *B. hominis* positive patients

		n=37	p
Gender, n (%)	Male	19 (51.4)	0.190
	Female	18 (48.6)	
Disease status, n (%)	Remission	20 (54.1)	0.349
	Active	17 (45.9)	
Site of involvement, n (%)	Pancolitis	14 (37.8)	0.003
	Left colon	19 (51.4)	
	Proctitis	4 (10.8)	
Total Mayo score, n (%)	Mild-moderate	11 (64.7)	<0.001
	Severe	6 (35.3)	

Table 5. Comparisons of the presence of *B. hominis* in different localization of ulcerative colitis separated as active or remission

		B. hominis		
		Negative (n=77)	Positive (n=37)	p
Pancolitis, n (%)	Remission	14 (63.6)	8 (36.4)	0.747
	Active	13 (68.4)	6 (31.6)	
Left colon, n (%)	Remission	15 (62.5)	9 (37.5)	0.973
	Active	17 (63)	10 (37)	
Proctitis, n (%)	Remission	13 (81.3)	3 (18.8)	0.999
	Active	5 (83.3)	1 (16.7)	

According to the results of the PCR examination, the patients were separated into two groups as *B. hominis* positive and *B. hominis* negative. The *B. hominis* prevalence was determined to be similar in the UC groups in remission and in acute flare-up (remission=32.2% vs. active=32.6%, p=0.961). There was no statistically significant difference between the groups in respect of age, gender, site of involvement, and hemoglobin, eosinophil, and CRP values (Table 3).

In the *B. hominis* positive group, there was no statistically significant relationship between *B. hominis* positivity and gender or disease activation status. The majority of the *B. hominis* positive patients were determined to have a Mayo score of mild-moderate severity (p<0.001). The most common site of involvement in the *B. hominis* positive patients was determined as left colon involvement (p=0.003) (Table 4). When we compared the presence of *B. hominis* in different localization of UC and the activation status, there was no statistically significant difference between the groups (Table 5).

DISCUSSION

As the microbiota of patients with IBD has been shown to be different from that of healthy individuals, the role of the microbiota in IBD pathogenesis has been revealed to be significant (2,4). Recent studies have shown that the microbiota varies according to the subtype of IBD and the activity phase. For example, *Fusobacterium* and *Escherichia* are much greater in CD than in UC, whereas *Methanobrevibacter*, *Anaerostipes*, and *Christensenella* species of unknown classification are much greater in UC than in CD (13,14). *Faecalibacterium prausnitzii* and *Roseburia hominis* that produce butyrate have been shown to be decreased in UC (15). In the microbiota of twins diagnosed with UC, Proteobacteria and Actinobacteria have been determined to increase in the acute flare-up period, whereas there is no difference in the microbiota in the remission period compared with the healthy control groups (16). In another study, it was determined that sulfate-reducing bacteria and facultative anaerobic bacteria increased in the active period, and *F. prausnitzii* and *Lactobacillus* species decreased (17).

As in those studies, the majority of studies that have investigated the relationship of IBD and dysbiosis have been conducted with bacteria that constitute >90% of the microbiota (18). However, more recently, there have been studies with other microorganisms, primarily parasites, and it has been shown that the microorganisms forming the microbiota interact with each other, and that microorganisms, to a lesser degree than flora, can also create big changes in the whole intestinal microbiota (11). In this respect, *Blastocystis* has come to prominence. Several studies have demonstrated that a change in *Blastocystis* affects bacterial flora (11,19). The effects of *Blastocystis* on the microbiota are significant, and because the clinical and pathological findings are similar to those of IBD, this suggests that it could play a role in the pathogenesis of IBD.

Blastocystis hominis is a parasite seen worldwide, which is spread via the fecal-oral route, especially in conditions of poor hygiene, and is more common in tropical and subtropical regions. *Blastocystis* spp. is a parasite that has been determined most often in epidemiological studies and is seen in almost all parts of the world (13). The prevalence can vary between countries and even between different communities within the same country. In developing countries, the prevalence has been reported as up to 60%, whereas in developed countries, the rate varies between 1.5% and 10% (20). Although this was not a prevalence study, the prevalence of *B. hominis* in all patients with UC was found to be 34%. Previous studies in Turkey have reported rates varying from 0.48% to 44.4% (20).

Microscopic methods (Lugol, Giemsa, and trichrome), culture, and molecular (PCR) methods are used in the diagnosis of *B. hominis*. As culture and PCR are more reliable methods, these methods are used more in clinical studies (21). Several diagnostic tests have been developed based on PCR for the diagnosis of *Blastocystis* spp. Most studies have shown the PCR method to be superior to staining applied directly to feces and culture methods (22). In a previous study, the PCR method was shown to have 100% specificity in the diagnosis of *Blastocystis* spp., and correct results have been obtained even when few parasites have been found in the feces, and when parasites are degenerated (23).

Although *Blastocystis* has been known for approximately 100 years, there is still doubt regarding its pathogenesis (12). It may cause simple symptoms, such as diarrhea, constipation, nausea, loss of appetite, and abdominal pain in most patients, and occasionally, it can lead to severe symptoms, such as fever and rectal bleeding (23). In studies investigating its pathogenesis, edema in the intestinal mucosa has been shown to cause inflammatory cell infiltration by the parasite penetrating the intestinal epithelial cells and the stimulated production of inflammatory cytokines, such as interleukin 8 (IL-8) and granulocyte-macrophage colony-stimulating factor (24). In addition, proteases that are expressed have been reported to play a role in infection by hydrolyzing connective tissue proteins, such as keratin, collagen, and secretory immunoglobulin A (25). It is thought that this inflammatory process and cytokine expression induced by *B. hominis* could be the trigger for the development of IBD (24).

In the present study that investigated the role of *B. hominis* in UC flare-up, the prevalence of *B. hominis* was determined to be similar in patients in the remission period and those in the active period. There are very few studies in the literature that have examined the relationship of *B. hominis* and UC. In a previous study conducted in our center, which used the native-Lugol and formol acetate concentration method, *B. hominis* prevalence was determined to be higher in patients with UC than in the healthy control group (patients with UC=8.7% vs. control group=3.1%, p=0.016) (13). However, in that study, the patients were compared with healthy individuals without any separation of activation or remission, and the native-Lugol and formol acetate concentration method was used, which has a low sensitivity (26).

Yamamoto et al. (27) performed protozoa screening of 215 patients with UC using the trichrome staining method, and *B. hominis* was determined at the highest rate. In addition, protozoa frequency was seen to be greater in persistent and intermittently active patients than in those in remission. In their study, the evaluation was not of the effect of the protozoa on disease activity but of the effect on activity frequency, and the trichrome staining method was used, which has a low sensitivity in protozoa diagnosis (28). In the study by Tai et al. (29), *B. hominis* was determined in fecal examinations and treatment-resistant symptoms in six patients with UC, which were reported to be completely resolved following 14 days of antibiotic treatment. As metronidazole that was given to the patients is effective against several parasites and bacteria in addition to *B. hominis*, it cannot be said whether or not *B. hominis* was the reason for the resistance to treatment. Nevertheless, from these findings, *B. hominis* can be considered to play a role in the pathogenesis of IBD.

In contrast to these studies, Coşkun et al. (30) examined *B. hominis* prevalence in the fecal cultures of 150 patients with UC and found it to be lower in active patients than in those in remission (active UC=3.8% vs. remission=11.8%, p<0.05). In their study, there were few active patients with UC (n=12), and the culture method was used, which has a lower sensitivity than PCR in the diagnosis of *B. hominis* (31). Peterson et al. (32) found the prevalence of *B. hominis* to be lower in patients with UC than in the healthy control group using the PCR method (UC=5% vs. control group=19%, p<0.05). In another study, Rossen et al. (33) screened for *B. hominis* using the triple fecal test method and reported lower prevalence in active patients with UC than in the healthy control group (UC=13.3% vs. control group=32.5%, p=0.014). The triple fecal test and PCR used in these studies are both methods with high sensitivity, but the number of patients was low (45 and 41 patients, respectively). As the prevalence of *B. hominis* in the current study was determined to be similar in the remission and active phase UC groups, this suggests that *B. hominis* does not play a role in UC flare-up.

As *B. hominis* prevalence has been found to be greater in the healthy groups than in patients with UC in most studies, this suggests that *B. hominis* has a protective role rather than being a cause of UC and could be a marker of a healthy microbiota (23,30,32,33). In a healthy microbiota, the butyrate produced by bacteria is used for ATP production with β-oxidation by colon epithelial cells. The oxygen used during this process reduces the oxygen concentration in the microbiota. Therefore, while oxygen concentration is low in a normal microbiota, when the microbiota is disrupted, the oxygen concentration increases (34). It has been shown that in patients with IBD, obligate anaerobes are decreased in the microbiota, and facultative anaerobes, such as Enterobacteriaceae, are increased (11). The lesser prevalence of the obligate anaerobe *Blastocystis* in IBD could be a result of this dysbiosis (11). In another study, beneficial bacteria, such as Clostridia, Ruminococcaceae, and Prevotellaceae, were determined less in *Blastocystis* negative patients than in *Blastocystis* positive patients, and Enterobacteriaceae were seen more, showing that this change triggered inflammation (35). In addition, *Blastocystis* has been shown to alleviate colitis symptoms by stimulating mucous production mediated by IL-22 (36). In the current study, the severity of the disease was found to be milder in those determined with *B. hominis*, and this finding supports the view that *B. hominis* may have a protective role.

Although *B. hominis* was detected mostly in patients with left colonic type UC, there was no significant difference when involvement site of UC, activity status, and the presence of *B. hominis* were evaluated together as seen in Table 4 and 5.

Of the studies in the literature investigating the relationship between UC and *B. hominis*, the current study included the highest number of patients. Another strong aspect of our study was the use of PCR, which is one of the most sensitive methods for the diagnosis of *B. hominis*. Although this was a single-center study and patients using drugs, such as antibiotics and probiotics, in the last 6 months were excluded, that patients were not questioned about the use of these types of drugs within the last year could be considered a limitation of the study.

Although *B. hominis* is a parasite frequently encountered in UC, the prevalence was found to be similar in both remission and active phases. Moreover, the disease was observed to be milder in the majority of the patients determined with *B. hominis*. In conclusion, *B. hominis* does not play a role in the activation of UC, but conversely, it could have a protective role. Nevertheless, there is a need for further, large-scale, comprehensive studies on this subject.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of the Antalya Training and Research Hospital.

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

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